RECIPES

Stains

2% neutral uranyl acetate (UA)

- light sensitive, alpha emitter
- wear gloves, cumulative toxin.

1. Make up 4% uranyl acetate in dH_2O (2g in 50ml)
2. Make up 0.3 M oxalic acid in dH_2O (1.9g in 50ml)
3. Take: 4% uranyl actate and 0.3 M oxalic acid and mix 1:1
4. This will give you 2% UA in 0.15 M oxalic acid
5. Add 25% ammonium hydroxide drop by drop to a pH of 7 or 8. Do this with constant stirring in the hood and check periodically with a pH stick. If you go over 7.5 or 8, a precipitate forms and you can start all over again.

Note: if the ammonium hydroxide is too old or has been opened too many times, the concentration of the ammonium changes with the result that you have to add too much ammonium to your solution and this will give you a neutral UA with less contrasting ability than if you use fresher ammonium solution.
Note: Undissolved uranyl acetate crystals will settle out, avoid disturbing these when removing aliquots.

5% alcoholic uranyl acetate (for *en bloc* staining)
Add 1.25g of UA to 12.5 ml of 100% alcohol, shake gently, the UA should dissolve instantly. Add 12.5 ml of dH_2O. Store at 4°C. The 100% alcohol used depends on whether the dehydration steps are to be done with a graded series of methanol or ethanol.

Reynold's lead citrate

Note: this stain reacts with CO_2 and carbonate causing it to precipitate. These contaminants should be avoided during preparation and long term storage.

1. Boil fresh 18.2 mega Ohm dH_2O for 10 minutes to remove any CO_2. Cover with Petri dish and cool for 30 minutes before use.
2. Make 10 ml of 1M NaOH using the freshly prepared water.

Note: NaOH will absorb atmospheric CO_2 over time, avoid using old stocks of NaOH pellets.

3. Add 30 ml of freshly prepared water to the 1.33g lead nitrate and 1.76g sodium citrate; shake/sonicate for 60 minutes to allow conversion of lead nitrate to citrate.
4. Add 8 ml of 1M NaOH; solution should clear, make up to 50 ml with freshly prepared water.
5. Store in foil-lined plastic container at 4°C. The stain is stable for up to 6 months; discard if it becomes cloudy.
6. Before use, microfuge aliquot at 5,000 rpm for 5 minutes.

Reagents for cryo-preservation and cryo-sectioning:

10% gelatin in 0.1M phosphate buffer, pH7.4

1. Add 5g gelatin powder (food grade) to 50ml 0.1M phosphate buffer, pH 7.4 in a 50 ml falcon tube.
2. Loosen the lid, place in a beaker and microwave carefully until hand hot (approx. 60˚C). Do not burn; do not boil.
3. Tighten lid and vortex until dissolved.
4. Centrifuge in the bench-top 3000rpm for 5min.
5. Transfer supernatant to clean falcon tube and add 100μl 10% sodium azide. Mix and store at 4˚C or at -20˚C.

2% methylcellulose (Sigma M-6385; methylcellulose, 25 centipoises)

For 200ml:

1. Bring 196 ml dH_2O to 90˚C, remove from heat and add 4 g methylcellulose.
2. Mix, then place in an ice bucket and stir slowly, covered with a plastic Petri dish, until the solution reaches a temperature of 10˚C.
3. Seal with Parafilm and leave stirring over night in the cold room.
4. Turn the stirring off on the following day and let the mixture “ripen” for 3 days before centrifuging.
5. Centrifuge for 95 min at 100,000*g*.
6. Decant the supernatant and store at 4°C. The solution is stable for at least 3 months, 0.02% sodium azide can be added to prevent microbial growth.

2.3M sucrose

Take a glass beaker, fill with 100ml 0.1 M phosphate buffer pH 7.4 and mark the meniscus on the beaker. Weigh 80g sucrose in the EMPTY glass beaker and fill with 0.1 M phosphate buffer pH 7.4 up to the mark for 100ml 2.3 M sucrose, stir until dissolved.

Methylcellulose/sucrose (MS)

Add equal amounts of 2% methyl cellulose and 2.3M sucrose together. Solution stable for at least 3 months if azide added.

15% PVP, 1.7M sucrose (PVP/sucrose)

For 20 ml 15% PVP (Polyvinylpyrrolidone):

4g PVP (Sigma PVP-10)

0.6 ml 1.1 M Na_2CO_3

17 ml 2 M sucrose in phosphate buffer, pH 7.35.

This can be made several ways. You can make a paste of the PVP with the Na_2CO_3 and add the sucrose, cover and leave overnight at room temperature, during which time tiny air bubbles in the paste escape into the air, leaving behind a clear solution. Store in aliquots at -20°C.

Buffers

10x KOAc stock

- 250mM HEPES 11.92g
- 1.15M KOAC 22.58g
- 25mM $MgCl_2$ 1.02g (5 ml of 1M)
- make up to 200 ml dH_2O (check pH 7.4)
- adjust pH to 7.4 (approx. 7 or 8 pellets of KOH)

Store at 4°C, excess can be stored at -20°C.

0.1M Phosphate buffer (PB)

0.1M PB is prepared by mixing x ml of 0.2M Na_2HPO_4 with y ml of 0.2M NaH_2PO_4. As described in the table below, the relative proportions of each phosphate solution determines the final pH.

pH (at 25°C)	x ml	y ml
5.8	4.0	46.0
6.0	6.15	43.85
6.2	9.25	40.75
6.4	13.25	36.75
6.6	18.75	31.25
7.0	30.5	19.5
7.2	36.0	14.0
7.4	40.5	9.5
7.6	43.5	6.5
7.8	45.75	4.25
8.0	47.35	2.65

2. Dilute to 100ml with dH_2O.

3. The osmolarity of PB can be adjusted with sucrose or varying the concentration of phosphates:
0.1M PB, pH 7.2 = 226 mOsM
+ 0.18M sucrose = 425 mOsM

0.05M PB = 118 mOsM
0.075M PB = 180 mOsM
0.15M PB = 350 mOsM

0.1M PB + glutaraldehyde
1.2% glut. = 370 mOsM
2.3% glut. = 490 mOsM
4.0% glut. = 685 mOsM

0.2M sodium cacodylate

Note: Prepare in fume hood, sodium cacodylate contains arsenic. Once diluted the solution is safe to handle although any spills must be removed immediately.

Dissolve 21.4g of sodium cacodylate in 500 ml of dH_2O. Store at 4°C.